Table 1

	Site (on Figure 8)	Motif
Protein Kinase C phosphorylation	8-10	SNK
	77-79	TPR
	118-120	THR
	203-205	TKK
	228-230	TAK
	311-313	STR
	312-314	TRK
	319-321	SNR
	384-386	STR
	403-405	SNR
	408-410	SSR
	409-411	SRR
Casein Kinase II phosphorylation	8-11	SNKE
	139-142	SDFE
	177-180	TGPD
	194-197	SEAE
	199-202	THLD
	224-227	TRDE
	228-231	TAKE
	238-241	SLVE
	325-328	TLNE
	423-426	SSSE
	425-428	SESD
	427-430	SDGD
CAMP- & cGMP-dependent protein kinase phosphorylation	405-408	RRFS
Tyrosine Kinase phosphorylation	40-47	KLHDQEEY
Myristoylation	16-21	GLRMSI
	143-148	GSGYTD
	119-224	GNTIGT
	266-271	GSQNAH
	291-296	GSSDAA
	315-320	GVDHSN
	389-394	GMPQGK
Amidation	370-373	HGRK
RGD	152-154	RGD
Gycosaminoglycan Attach. Site	161-165	SGDG
Asu-Glycosylation	382-386	NNST
	383-387	NSTR

A key feature of the protein is a cell attachment sequence at residues 152-154 (RGD). The Arg-Gly-Asp sequence plays a role in receptor interactions in general, and in fibronectin is essential for cell surface receptor binding to a specific integrin. More notable is the presence of this motif in some forms of collagens (bone matrix protein), fibrinogen, vitronectin, von Willebrand factor (VWF), snake disintegrins, and slime mould discoidins. It is highly probable that this part of the phosphatonin is involved in receptor and/or bone mineral matrix interactions. Also these interactions mediate the following:

1. osteoid mineralization (osteoblasts).
2. Na-dependent phosphate co-transporter gene expression regulation.
3. 24 hydroxylase and/or 1 alpha hydroxylase gene expression regulation (kidney).
4. bone and dental mineral matrix interactions and regulation of mineral deposition via nucleation.

The presence of a glycosaminoglycan attachment sequence at residues 161-164 (SGDG), has important implications concerning bone mineral attachment and interactions. The role of proteoglycans in bone is well documented particularly in cell signaling. It is highly probable that this part of the molecule is also essential for the above bioactivities (point 1 to 4), and in particular osteoblast mediated mineralization of osteoid.

The RGD motif is in a region of predicted turn (Garnier prediction Antheprot), and is flanked by two regions of β-sheet (residues 134 to 141 and 172 to 178). The predicted sheet structure is in turn flanked by two regions of extended α-helix (121 to 132 and 196 to 201). The general structural context, predicted turn and presence of the RGD cell attachment sequence is similar to that found in osteopontin. The protein also has a number of predicted phosphorylation motifs for protein kinase C, casein kinase II, tyrosine kinase, and cAMP cGMP-dependent protein kinase. MEPE was also found to have a large number of N-myristoylation sites, and these sites appear to be a feature of RGD containing phospho glycoproteins (osteopontin, vitronectin, collagen, h-integrin binding protein, dentin-sialophosphoprotein, dentin-matrix-protein-1, bone-sialoprotein-II

and fibronectin). There is an unusually high content of aspartate, serine and glutamate residues (26%), as in osteopontin (37%). Of particular interest is the complete absence of cysteine residues in MEPE sequence, indicating that cysteine-cysteine disulphide bridges do not play a role in the secondary structure of this molecule. Sequence homology to dentin phosphoryn (DPP) was found after screening the trembl database with MEPE. A region at the C-terminus of MEPE has a sequence of aspartate and serine residues (residues 414-427) that are almost identical (80% homology), to a recurring motif found in DPP) (figure 26A and 26B). Physicohemical comparison of the MEPE motif (DDSSESSDSGSSSESD) with the DSP motif (SDSSDSSDSSSSSDSS), increases the homology to 93%. The MEPE-motif occurs once at the C-terminus in MEPE (residues 414 to 427), whereas the DSP homologue is repeated at DSP residue positions 686 to 699, 636 to 646, and 663 to 677. Moreover, two related sequences DSSDSSDSNSSSDS and DSSDSSDSSNSSDS, also with 80% homology to the MEPE-motif are found in DSP at positions 576 to 589 and 800 to 813 respectively. A similar motif with 60% homology (DDSHQSDESHHSDESD), is also found in osteopontin (residues 101 to 116), and a casein kinase II phosphorylation site is contained within the region of homology (figure 12). Skeletal casein kinase II activity is defective in X-linked rickets (Rifas, loc. cit.). Although the osteopontin MEPE-motif is central and not C-terminal, cleavage of osteopontin in vivo has been reported and this would generate a peptide with the MEPE motif placed C-terminal (Smith, J. Biol. Chem. 271 (1996), 28485-28491). Additional sequence homology to the C-terminal MEPE-motif is also found in DMA-1 at residues 408 to 429 (SSRRRDDSSESSDSGSSSESDG). A graphical presentation of the regional sequence homology of the MEPE-motif in DSSP, DMA-1 and OPN is presented in figure 12 as a 'Ilanview' statistical plot, and Table 2 presents the sequence similarities in alignment.

Table 2

MEPE versus DSSP

Upper sequence MEPE:

414	DSSESSDSGSSSES	427	(SEQ ID NO: 7)
686	DSSDSSDSSSSSDS	699	(SEQ ID NO: 13)